and were then screened by PCR for acquisition of a higher molecular mass product, cph1:hisG. This process of transformation and 5-fluorocytosine treatment was repeated to disrupt the second copy of CPH1. cph1/cph1 isolates were screened by PCR for complete loss of the wild-type CPH1 PCR fragment.

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### TECHNICAL COMMENTS

**The Entropic Cost of Binding Water to Proteins**

In a recent Perspective (1) about the entropic cost of binding water molecules to proteins and other macromolecules, Jack D. Dunitz uses thermodynamic data on water, anhydrous salts and their hydrates to set limits on the entropy decrease for transferring a water molecule from liquid water to the macromolecule. The limits set were 0 to 7 cal mol⁻¹ K⁻¹ with larger entropy decreases corresponding to more tightly held waters. Dunitz also states that thermodynamic data from which these entropy changes can be directly calculated are "nonexistent." Such data do exist and calculations of these entropy changes have been reported in the literature.

Data for calculating ∆S/∆n, the entropy change occurring when a mole of water is transferred from liquid water to solid protein, as a function of n, the moles of water bound per mole of protein, can be obtained from measurements of sorption isotherms of water vapor on solid proteins at several temperatures (2). Isotherms of water vapor on proteins generally exhibit hysteresis, but it has been shown that correct entropy calculations can be made even in the absence of isotherm reversibility (3). For example, calculations of ∆S/∆n values for water bound to native ovalbumin (2) have been made on data from the literature (4). The variation of such entropies with n has also been discussed (5). Values of ∆S/∆n for ovalbumin varied from approximately 0 to 12 cal mol⁻¹ K⁻¹ for absorption isotherms and from approximately 0 to 20 cal mol⁻¹ K⁻¹ for desorption isotherms. Larger entropy decreases were generally seen at lower values of n. The fact that some of these entropy decreases are greater than the estimated limit of 7 cal mol⁻¹ K⁻¹ suggests that the binding of more tightly held waters to a protein can cause a decrease in protein entropy as well as a decrease in water entropy. Thus, uptake of water could lead to fewer, or more ordered protein conformations, or both.

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**REFERENCES**

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**Dating Hominid Sites in Indonesia**

C. C. Swisher et al. (1) recently published two new dates for hominid sites in Java based on ⁴⁰Ar/³⁹Ar laser-incremental heating analyses. They propose mean-weighted ages of 1.81 ± 0.04 million years ago (Ma) for the Mojokerto and 1.66 ± 0.04 Ma for the Sangiran site. On the basis of these dates they draw far-reaching conclusions about the early migration of the ancestor of Homo erectus out of Africa as well as an explanation for the absence of the Acheulean stone tool culture in Asia. These new ⁴⁰Ar/³⁹Ar ages are based on hornblende separated from pumice recovered at Sangiran and Mojokerto. However, the geological context of these hominide samples is not clear, and the new ages are contradicted by a wide range of established data.

A discrepancy of about 0.9 Ma between the ⁴⁰Ar/³⁹Ar ages (1.81 and 1.66 Ma) given by Swisher et al. and the existing magnetostatigraphy [which is based on detailed sections of Sangiran (0.97 to 0.73 Ma) and Mojokerto (0.97 Ma) reported by Hyodo et al. (2) in 1993] is not adequately explained by Swisher et al. The Hyodo et al. (2) magnetostatigraphy, based on a solid lithostatigraphy (3), corroborates perfectly with a series of fission track ages (4) indicating dates all less than 1.0 Ma. In this light, the geological context of samples which yielded the older dates must be critically reviewed.

At the Mojokerto site the pumice was taken from a conglomeratic volcanic sandstone, which invites the interpretation that the pumice was likely reworked and redeposited. Swisher et al. state, about the Sangiran sample, that the pumice was handpicked from a volcanic pumice-rich layer. There is inadequate information about the lithostratigraphy and exact stratigraphic position of this sample in the Sangiran section and about the relationship of the volcanic pumice-rich layer to the high number of well-described and recognizable tuff layers in the Sangiran area of which some have fission track data (3).

There is agreement between the normal polarity found at the Mojokerto site by Swisher et al. (1) and that reported by Hyodo et al. (2), but, on the basis of the ⁴⁰Ar/³⁹Ar age of 1.8 ± 0.04, Swisher et al. place this site in the Olduvai event. On the basis of the paleomagnetic properties of the section in Sangiran as well as in Mojokerto, Hyodo et al. (2) demonstrate that the normal polarity of these sites represent the Jaramillo event, which suggests an age of approximately 0.97 Ma. We see no reason to doubt this paleomagnetic sequence, which is also corroborated by fission track ages (4). In addition to the discrepancy of the new ⁴⁰Ar/³⁹Ar ages compared with the paleomagnetic and fission track data, the biostatigraphy of Sangiran and Mojokerto (5) contradict the newly proposed ages for these sites. Trinil, which contains the type specimen of Homo erectus discovered by Dubois (6) and is characterized by Stegodon, is widely considered to have an age of about 1 Ma. The Kedung Brubus fauna, characterized by new arrivals of the Asiatic mainland, like Elephas, to which the Mojokerto fauna belongs, is younger (5) than the Trinil fauna based on all key paleomagnetic markers (5).

The ⁴⁰Ar/³⁹Ar dates of Swisher et al. may themselves be "technically correct," but until their geological context is established, it is premature to attach such far reaching conclusions to these new age estimates for the hominid of Java.

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